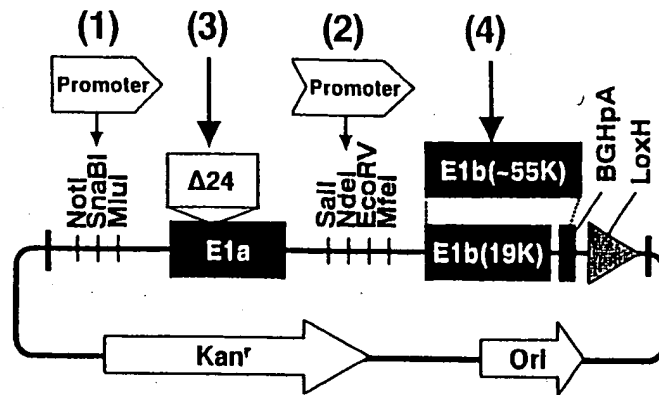
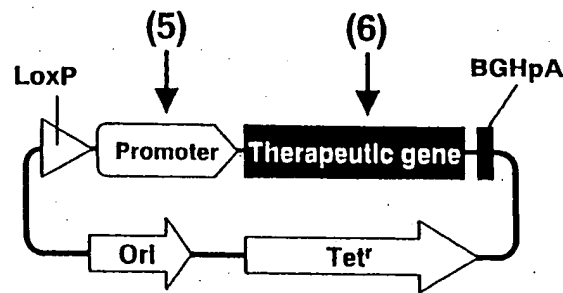


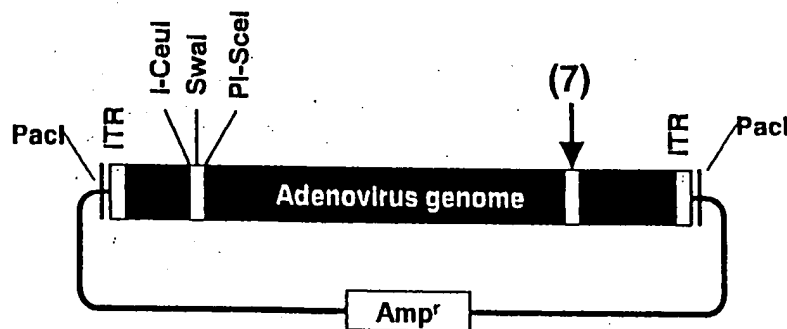
Applicant(s): Kenichiro Kosai et al.

METHOD OF PREPARING A PROLIFERATION-REGULATED
RECOMBINANT ADENOVIRAL VECTOR EFFICIENTLY AND
KIT FOR PREPARING THE SAME

A. Vector plasmid having a proliferation-regulating unit



B. Vector plasmid having a therapeutic gene-expressing unit



C. Adenoviral vector plasmid

Fig. 1

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METHOD OF PREPARING A PROLIFERATION-REGULATED
RECOMBINANT ADENOVIRAL VECTOR EFFICIENTLY AND
KIT FOR PREPARING THE SAME

Primer name	DNA sequence
S-E1A	5'-TCAGTCGCATCGCGCGCGCTACGTAACGGTTACCCGGTGAGTTCCTCAAGAGGC-3' Stuffer SphI NotI SnaBI MluI Ad5 474~497
AS-E1A	5'-GGACGTCCTAGGGTCGACGCCCCCATTTAACACGCCCATGTGCAAG-3' Stuffer AvrII SalI Ad5 1635~1658 (AS)
S-E1B19K	5'-TCAGTCCCTAGGGTCGACCATATGGATATCCAAATTGCGTGGGCTAATCTTGGTTACATCT-3' Stuffer AvrII SalI NdeI EcoRV MfeI Ad5 1684~1707
AS-E1B19K	5'-GGACGTGGATCCGCGTCTCAGTTCTGGATACAGTTC-3' <PCR condition> Stuffer BamHI Ad5 2262~2285 (AS) Thermal denaturation 94°C, 30 seconds Annealing 57°C, 30 seconds Elongation reaction 74°C, 60 seconds 30 cycles
S-BGHpA	5'-TCAGTCGGATCCGCATGCATCTAGAGCTCGCTGATC-3' Stuffer BamHI pRc/RSV 693~716
AS-BGHpA	5'-GGACGTGAATTCATAACTTCGTATAATGTATGCTATATGAGGTAATTCAGAAGCCATAGAGCCACCGCA-3' Stuffer EcoRI LoxH (AS) pRc/RSV 933~956 (AS)

Fig. 2

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 METHOD OF PREPARING A PROLIFERATION-REGULATED
 RECOMBINANT ADENOVIRAL VECTOR EFFICIENTLY AND
 KIT FOR PREPARING THE SAME

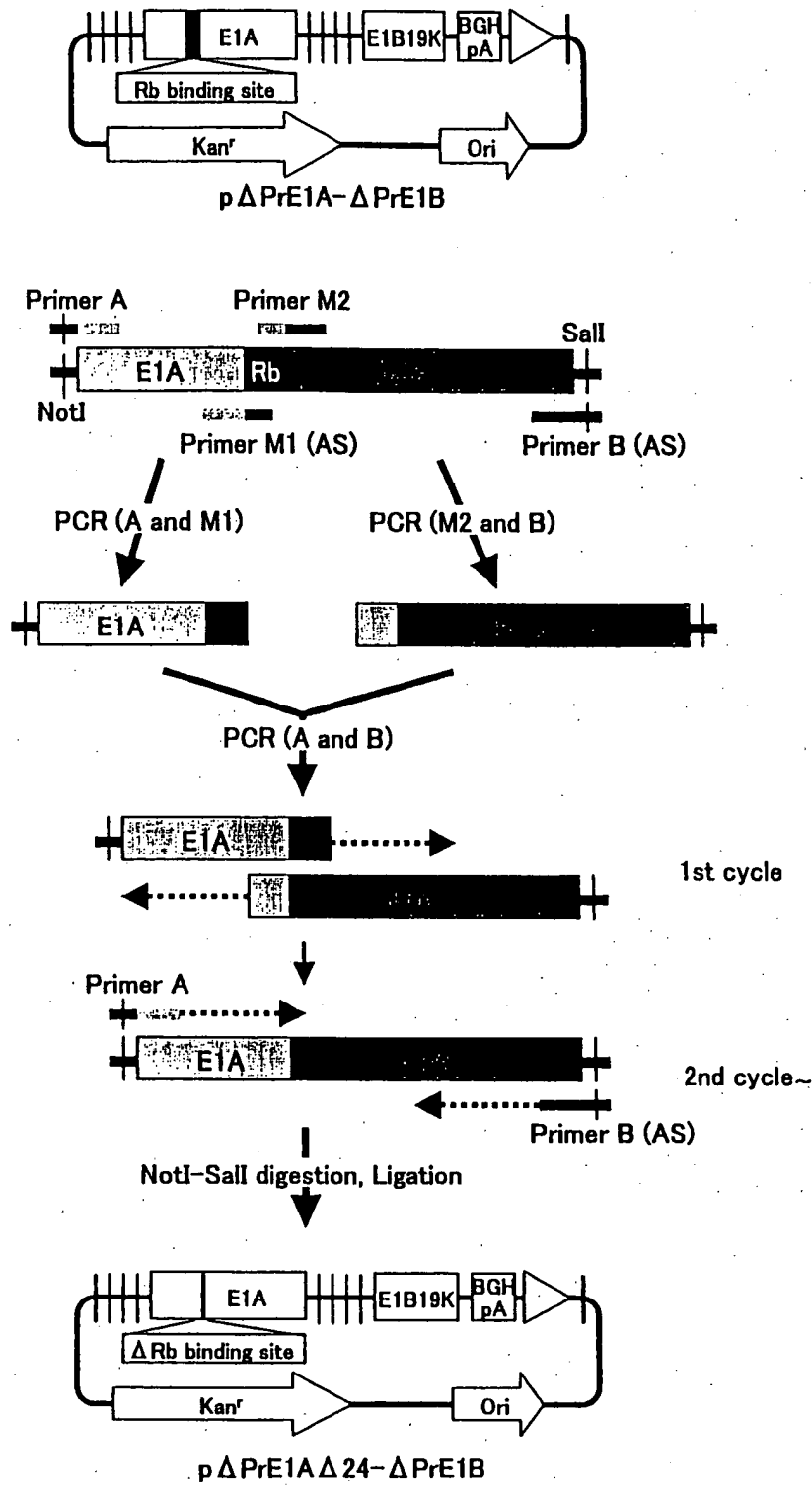


Fig. 3

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Primer name	DNA sequence
S-Δ24	5'-TTGTACCGGAGGTGATCGATCCACCCAGT-3' Ad5 903~922 Ad5 947~956
AS-Δ24	5'-TCCTCGTCGTCACCTGGGTGGATCGATCACC-3' Ad5 966~947 (AS) Ad5 922~913 (AS) <PCR condition> Thermal denaturation 94°C, 30 seconds Annealing 57°C, 30 seconds Elongation reaction 74°C, 60 seconds 30 cycles
S-E1B-2015	5'ATAAATGGAGCGAAGAAACC 3' Ad5 2015~2034
AS-E1B-4073	5' GGACGTGAATTCATAACTTCGTATAATGTATGCTATATGAGGTAATCTTGATCCAAATCCAAACAGAGTC 3' Stuffer EcoRI LoxH (AS) Ad5 4050~4073 (AS)

Fig. 4

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METHOD OF PREPARING A PROLIFERATION-REGULATED
RECOMBINANT ADENOVIRAL VECTOR EFFICIENTLY AND
KIT FOR PREPARING THE SAME

Primer name	DNA sequence
S-CMVp	5'-TCAGTCGTCGACCGTTGACATTGATTATTGAC-3' Stuffer SalI pRc/CMV 231~250
AS-CMVp	5'-GGACGTCAATTGGCTTGGGTCTCCCTATAGTG-3' Stuffer MfeI pRc/CMV 874~893 (AS)
S-CEAp	5'-TCAGTCGCGCGCATCATCCACCTTCCCAGAG-3' Stuffer NotI CEAp (-424~-405)
AS-CEAp	5'-GGACGTACGCGTCCAGGTCTCTGTGCTGTGC-3' Stuffer MluI CEAp (AS, -19~+1)
S-OCp	5'-CTGCAGGGTCAGGAGGAGAA-3' OCp (-834~-815)
AS-OCp	5'-GCGCTGGGCTGCTGCTCAGG-3' OCp (+12~+31)
<PCR condition> Thermal denaturation 94°C, 30 seconds Annealing 57°C, 30 seconds Elongation reaction 74°C, 60 seconds 30 cycles	

Fig. 5

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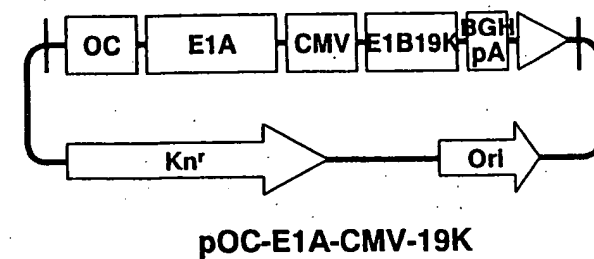
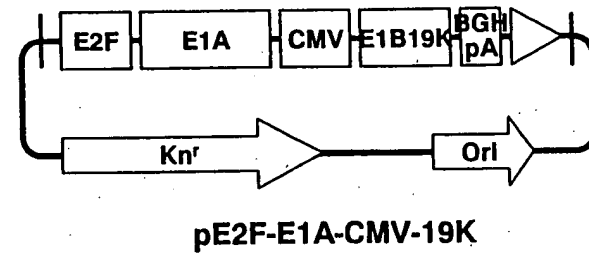
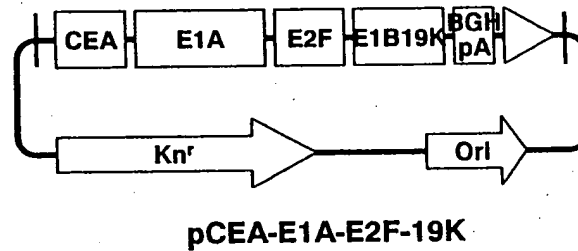
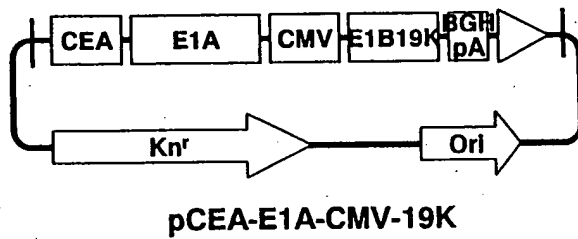
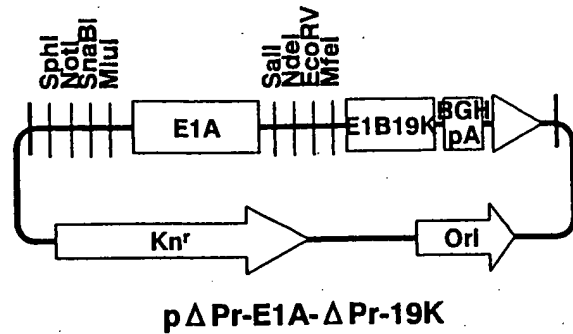
METHOD OF PREPARING A PROLIFERATION-REGULATED
RECOMBINANT ADENOVIRAL VECTOR EFFICIENTLY AND
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Fig. 6

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METHOD OF PREPARING A PROLIFERATION-REGULATED
RECOMBINANT ADENOVIRAL VECTOR EFFICIENTLY AND
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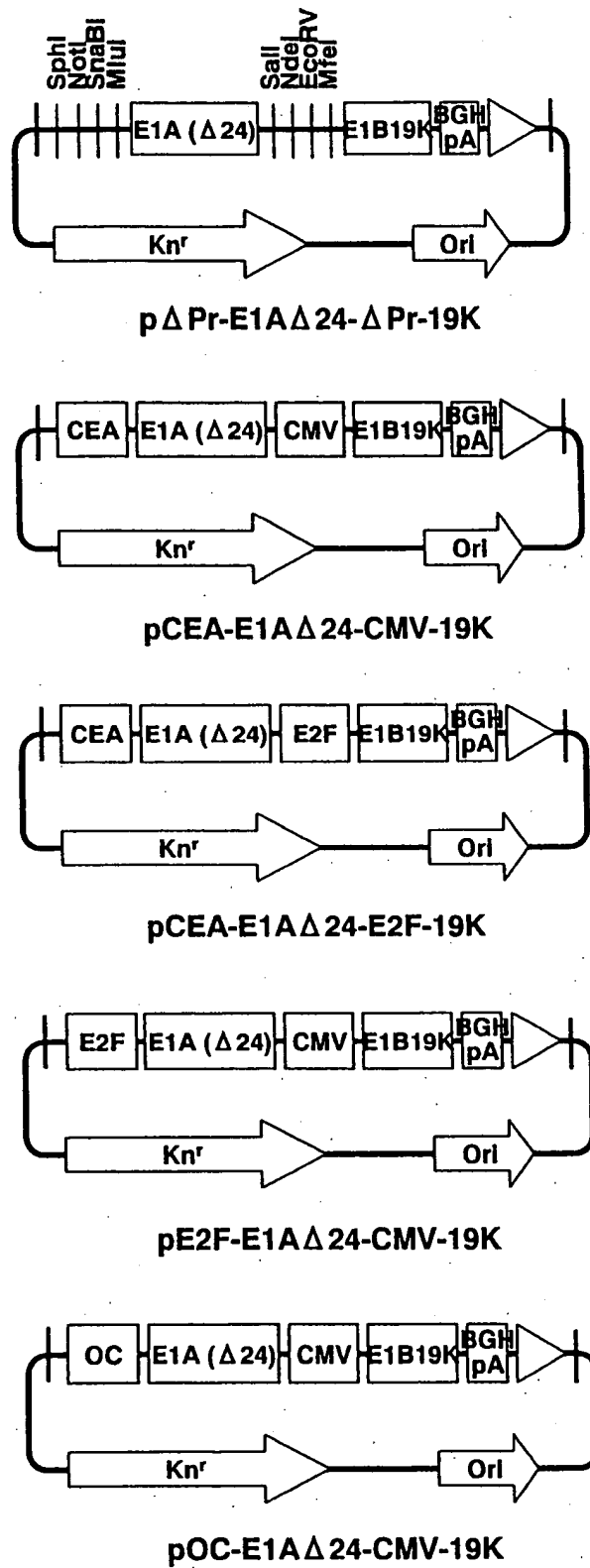
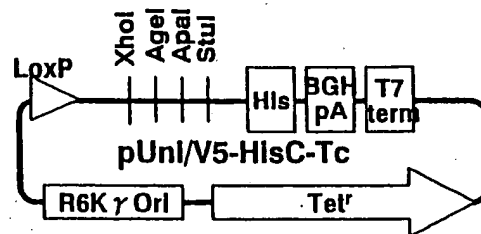


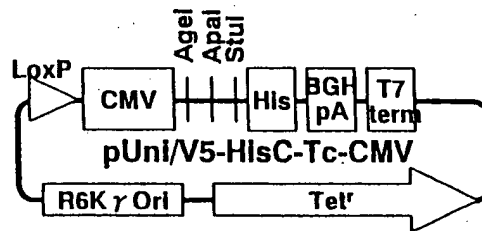
Fig. 7

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Vector plasmid having a therapeutic gene-expressing unit



First therapeutic gene-expressing vector plasmid
(having an integrated constitutive high-expression promoter)



First therapeutic gene-expressing vector plasmid

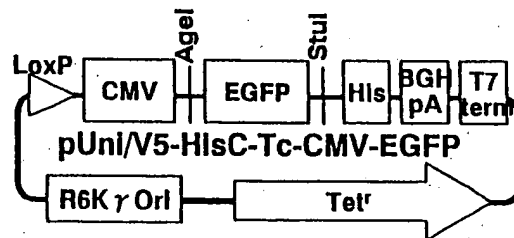


Fig. 8

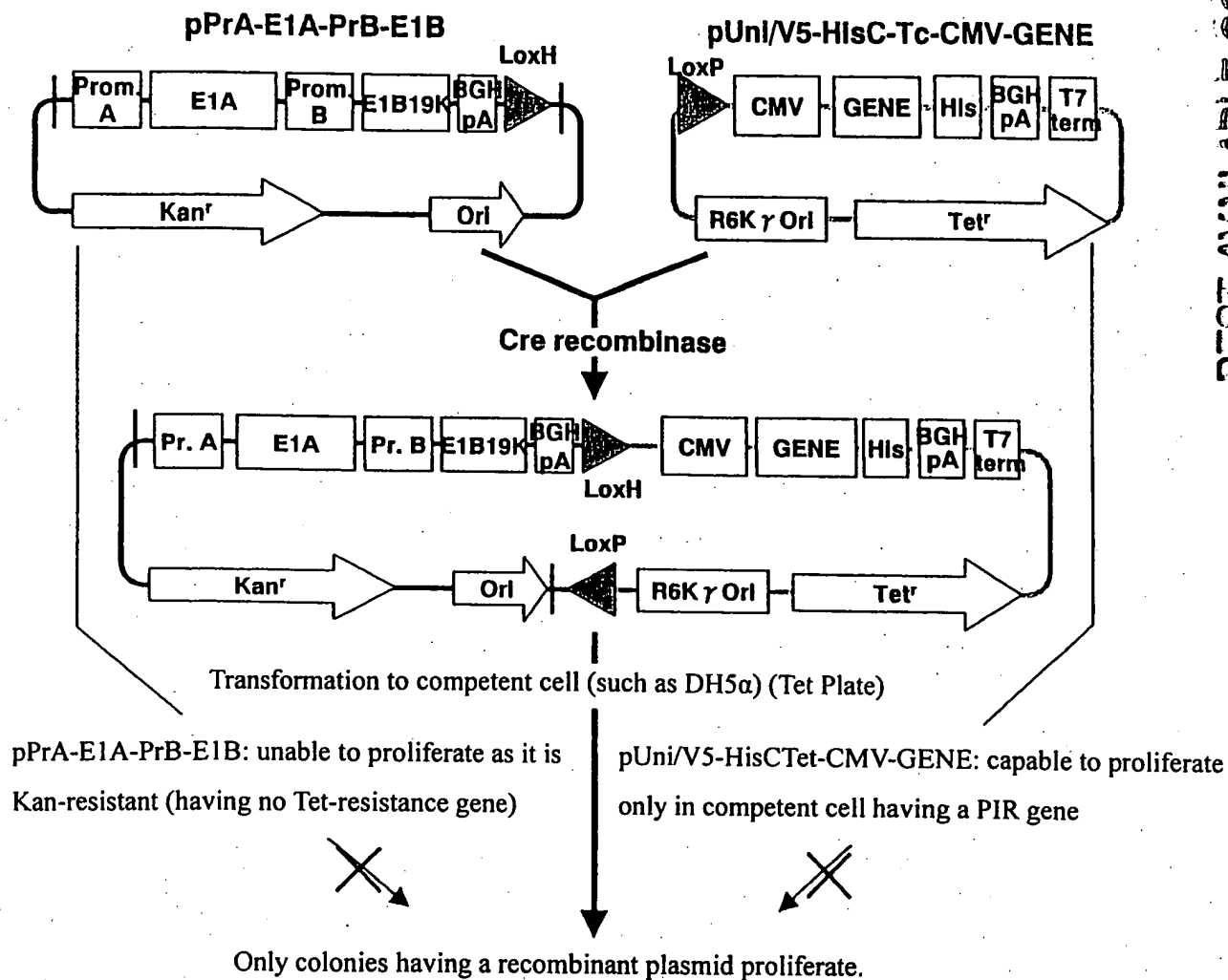


Fig. 9